Countryside biogeography: use of human-dominated habitats by the avifauna of southern Costa Rica. Ecologic. Appl. *11*, 1–13.
13. Sekercioglu, C.H., Loarie, S.R., Oviedo Brenes, F., Ehrlich, P.R., and Daily, G.C. (2007). Persistence of forest birds in the Costa Rican agricultural countryside. Conserv. Biol. *21*, in press.
14. Sekercioglu, C.H., Daily, G.C., and Ehrlich, P.R. (2004). Ecosystem consequences of bird declines. Proc. Natl. Acad. Sci. USA *101*, 18042–18047.
15. Stouffer, P.C. (2007). Density, territory size, and long-term spatial dynamics of a guild of terrestrial insectivorous birds near Manaus, Brazil. Auk. *124*, 292–306.
16. Partridge, L.W., Britton, N.F., and Franks, N.R. (1996). Army ant population dynamics: The effects of habitat quality and reserve size on population size and time to extinction. Proc. R. Soc. Lond. B. *263*, 735–741.
17. Powell, G.V.N., and Bjork, R.D. (2004). Habitat linkages and the conservation of tropical biodiversity as indicated by seasonal migrations of three-wattled bellbirds. Conserv. Biol. *18*, 500–509.
18. Sekercioglu, C.H. (2006). Increasing awareness of avian ecological function. Trends Ecol. Evol. *21*, 464–471.
19. Sekercioglu, C.H. (2006). Ecological contributions of bird populations. In Handbook of the Birds of the World, *Volume 11*, J. del Hoyo, A. Elliott, and D.A. Christie, eds. (Barcelona: Lynx Edicions), pp. 15–51.
20. Gradwohl, J., and Greenberg, R. (1982). The effect of a single species of avian predator on the arthropods of aerial leaf litter. Ecology *63*, 581–583.

Stanford University, Department of Biological Sciences, 371 Serra Mall, Stanford, California 94305-5020, USA.
E-mail: cagan@stanford.edu

DOI: 10.1016/j.cub.2007.02.019

# Cell Death: Hook, Line and Linker

**The programmed death of particular cells in *Caenorhabditis elegans* and *Drosophila* has been shown to occur by non-apoptotic programs regulated by developmental timing. Such alternative programs may be used as a general mechanism to eliminate differentiated, functional cells.**

**Ashish Kumar and Joel H. Rothman**

> "*An oil lamp may be extinguished owing to any of the following four causes — the exhaustion of the wick, the exhaustion of oil, simultaneous exhaustion of both wick and oil, or some extraneous cause like the gust of a wind. So may death be due to any of the forgoing four causes.*"
>
> Buddha, on the doctrine of Dependent Origination

The development and maintenance of tissues in animals depends not only on the ability of cells to reproduce, but also on their capacity for self-destruction when they become superfluous, damaged, or otherwise harmful [1]. In 1871, Virchow coined the term 'necrosis' as a general description for cell death. The widespread occurrence and biological relevance of an active cell death program was recognized a century later by Kerr, Wyllie, and Currie [2], who proposed a morphology-based classification of cell death into two categories: 'necrosis', which is restricted to rapid, violent and passive cell death caused by environmental perturbation; and 'apoptosis' or active programmed cell death, which occurs in natural and certain pathological situations.

A molecular hallmark of the latter process of apoptosis is activation of a cascade of proteases called caspases which cleave a variety of cellular targets, leading to cell death with distinctive morphological characteristics [2,3]. While apoptosis is the most common form of programmed death, it has become apparent that alternative programs can lead to other forms of cell death. Evidence has been accumulating that the apoptosis–necrosis dichotomy is insufficient to encompass the observed spectrum of morphological end-points [4]. Adding to the complexity is the unanticipated finding that, while inhibition of caspase activation can block apoptosis, it does not necessarily protect against cell death [5]. Rather caspase inhibition can reveal, or enhance, alternative caspase-independent cell death processes.

Recent findings by Abraham *et al.* [6] on the male linker cell in the nematode *Caenorhabditis elegans* and by Mazzalupo *et al.* [7] on nurse cells in the fruitfly *Drosophila* show that some types of natural, developmental death in these ecdysozoans occur by non-apoptotic programs. The *C. elegans* linker cell is born during an early larval stage and is essential for development of the male gonad. The *Drosophila* nurse cells are germ-line-derived cells that support oocyte development. The two papers provide strong evidence that caspases, and likely apoptosis, do not play a role in the natural developmental programs for linker and nurse cell deaths. Indeed, Abraham *et al.* [6] show that the dying linker cell displays a non-apoptotic morphology that is unexpectedly reminiscent of a previously described rare type of developmental cell death.

Since the original classification by Kerr *et al.* [2], three major morphologically distinct types of programmed cell deaths have been described [8] (Figure 1A). Type 1 is apoptotic cell death, characterized by cell shrinkage and extensive chromatin condensation. Formation of autophagic vacuoles inside the dying cell is typical of autophagic or type 2 cell death. Type 3 death is characterized by cellular swelling, often accompanied by "dilation of ER, nuclear envelope, Golgi and sometimes mitochondria, forming 'empty' spaces" [8].

An important criticism of the concept of alternative type 2 and 3 cell death programs is that they primarily seem to provide backup suicide mechanisms when the canonical apoptotic machinery is lacking or inhibited [4,9]. Are such 'non-canonical' cell death mechanisms relevant during normal development? The existence of non-apoptotic cell death in *C. elegans* [6] and *Drosophila* [7] as parts of natural developmental programs provides a definitive link between *in vivo* and *in vitro* data supporting the biological significance of alternative cell death programs.

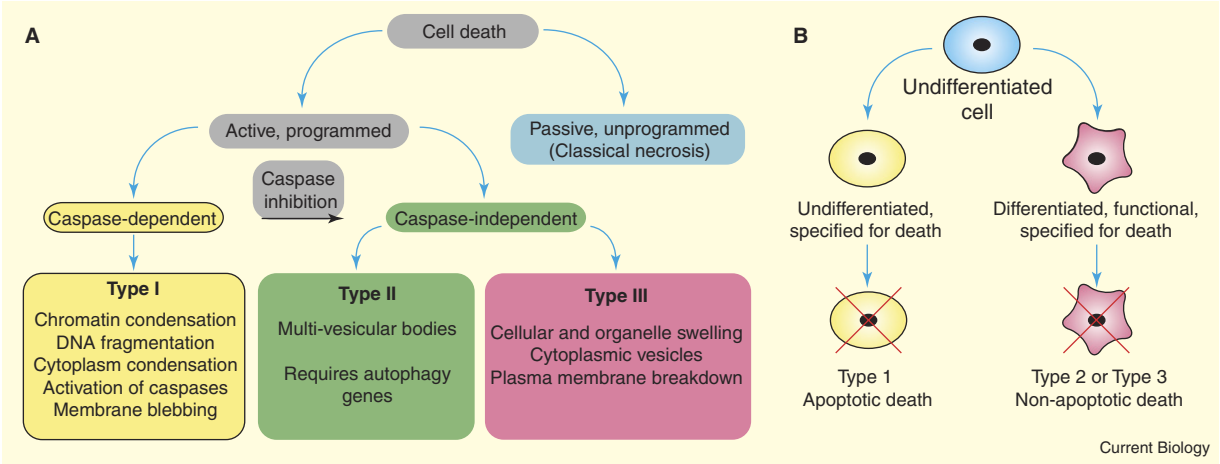


Figure 1. Diversity of cell death programs.

(A) Functional and morphological classification of cell death types. Programmed cell death is a genetically directed, active form of cell suicide, whereas classical necrosis is unprogrammed, accidental cell death. Programmed cell death is further subdivided according to its dependence on caspases. Type 1 cell death is apoptosis, promoted by caspase activation, and is the most common form in animal development. Type 2 and 3 cell death programs are frequently revealed after caspase inhibition of cells in which the cell death program has been activated, but also occur during normal development. Type 2, or autophagic cell death, is usually caspase-independent and requires autophagy genes. Type 3 is morphologically similar to classical necrosis but is programmed and occurs under normal physiological conditions. The male linker cell in *C. elegans* dies using an apparent Type 3 program [6]. (B) Model for the occurrence of non-apoptotic death. Type 2 and type 3 programs may be more prominent in cells that have differentiated and performed an active function. The linker cell of *C. elegans*, and the nurse cells and obsolete larval tissue cells in *Drosophila* are differentiated and functional before dying non-apoptotically.

The first evidence for a genetic basis for programmed cell death in animals came from studies in *C. elegans* [10]. During ontogeny of this worm, many somatic cells undergo an invariant pattern of programmed death at precisely defined lineal positions and times. It was the discovery of this predictable cell death pattern that proved that many animal cells possess a 'hardwired' suicide program. A conserved apoptotic machinery in *C. elegans* regulates nearly all somatic cell deaths, as well as the indeterminate cell deaths that occurs in the germline [10]. The genetic pathway controlling this process involves the CED-3 caspase and a number of other components required for its activation in dying cells and inhibition in survivors.

Nearly all cells in *C. elegans* that are destined to die undergo the death program within minutes of their birth by the type 1 program, before they have performed any function. In contrast, the linker cell lives much longer and carries out a critical function prior to its death. After its birth, this cell undergoes a complex path of migration in the male, leading the rest of the gonad, which develops behind it [11]. At the end of its migration, the linker cell dies during or just after the larval/adult molt and the gonad fuses with an exit channel, linking it to the external environment. Linker cell death in *C. elegans* must be tightly regulated to ensure male fertility.

By what death program does the linker cell die? While it was previously reported that linker cell death was partially dependent on CED-3 caspase function [12], Abraham *et al.* [6] convincingly show that linker cell death is fully CED-3-independent. Using GFP-fusion reporters to distinguish the linker cell from nearby cells unambiguously throughout and after the larval/adult molt, Abraham *et al.* [6] observed that linker cell death occurs reproducibly even in animals apparently lacking all caspase function [6]. They further show that linker cell death is independent of all previously known core cell death genes, including the proapoptotic *egl-1* and *ced-4* genes and the anti-apoptotic *ced-9* gene [6]. Their results provide strong evidence that linker cell death is governed by a non-apoptotic cell death program.

Like the *C. elegans* linker cell, the *Drosophila* nurse cells are also differentiated cells that die after completing a specialized function [13]. During oogenesis, the nurse cells transport maternal components to the oocyte, and ultimately expel their cytoplasmic content into the oocyte, after which the nurse cell remnants die. Mazzalupo *et al.* [7] observed no evidence of caspase activation in the cytoplasm before or during nurse cell death. Moreover, the expression of caspase inhibitors had no effect on nurse cell death. These findings led Mazzalupo *et al.* [7] to suggest that caspases play no role in the programmed nurse cell death, similar to the observations of Abraham *et al.* [6] for linker cell death.

If not apoptosis, then what mechanisms do the linker cell and nurse cells use to program their death? The alternative type 2 autophagic and type 3 necrosis-like programs are likely candidates (Figure 1A). Type 2 autophagic cell death, however, was discounted by both Abraham *et al.* [6] and Mazzalupo *et al.* [7], given the lack of increase in the abundance of autophagic protein-GFP fusions.

Further, Abraham *et al.* [6] observed that linker cell death is morphologically distinct from autophagic cell death and mutations in autophagy genes had no effect on linker cell death. Electron microscopy revealed that the morphology of linker cell death is strikingly similar to type 3 cell death. Similar morphological features have been reported previously in normally developing chick ciliary ganglion cells, axotomized chick retinal ganglion cells, and chick spinal cord motor neurons, leading Abraham *et al.* [6] to suggest that the distinctive morphology of the linker cell death program may be conserved in vertebrates.

Nurse cell death in *Drosophila* was previously considered apoptotic, based on the observation of chromatin condensation and DNA laddering [14]. But vacuoles have also been reported to be present in the nurse cells [15]. These observations imply a potential overlap between type 1-like and type 3 morphologies. Confirmation of nurse cell death morphology will require further ultrastructural analysis.

To ensure coordination between accomplishment of function and self-destruction, the non-apoptotic cell death programs in the linker cell and nurse cells must be tightly coupled to the completion of their respective functions. One possible way to mark the completion of linker cell function is through spatial cues. Past findings suggested that linker cell death may depend on the presence of its neighboring engulfing cell [16] and thus could be termed a 'murder'. Abraham *et al.* [6] assessed linker cell death in animals following ablation of the engulfing cell or in mutants defective for normal linker cell migration. Their results indicate that linker cell death does not apparently depend on engulfment or local signals, but instead is likely controlled by a cell-autonomous program.

If spatial cues do not regulate the death of the linker cell, then how does the cell know that it has completed its migration and needs to die? One possibility is that the cell death cue is temporally regulated. In fact, Abraham *et al.* [6] found that the linker cell does not die in animals with mutations in the *lin-29* and *let-7* genes, which control developmental timing at late stages, suggesting that it is temporal cues that regulate this death. In *Drosophila*, developmental timing signals are controlled by pulses of the steroid hormone ecdysone [17]. During metamorphosis, ecdysone activates programmed cell death to destroy obsolete larval tissues, including anterior muscles, larval midgut, and larval salivary glands, by a two-step transcriptional switch [17]. The developmental timing pathway has also been implicated in nurse cell death [18]. A comparative study of developmental timing pathways that regulate cell death in *C. elegans* and *Drosophila* might provide useful insights into the molecular signaling required for precise temporal control of cell death. In turn, such studies may lead to self-annihilating therapeutic 'nurse' cells or drug-delivery systems.

The features of type 3 cell death have traditionally been associated with necrosis, a passive form of cell death more similar to a train wreck than to programmed suicide [4]. But a number of recent reports have shown that type 3 cell death, variously referred to as programmed necrosis, necrosis-like program, oncosis, paraptosis and necroptosis, can occur under normal physiological conditions and during development [4–9]. These cases, including that of the linker cell death, are significant because they imply that cellular signaling pathways initiate type 3 death in response to specific triggers rather than by accident.

The case of *Drosophila* nurse cell death suggests that various routes of cell death may overlap or integrate, displaying multiple characteristics simultaneously. It is becoming increasingly clear that various types of cell death can share pathways of execution. Mitochondria, death receptors, lysosomes and endoplasmic reticulum may engage in a crosstalk leading to a continuum of diverse cell death programs. Such a spectrum of cell death programs may allow a variety of cellular responses to various circumstances or stimuli.

Why do the linker cell and the nurse cells choose to die through non-apoptotic pathways? Spatiotemporal coordinates, cell-size, cellular age, differentiation status and functional requirements may be important in regulating which death program is used by the cell. An appealing possibility is that non-apoptotic programs are prominent in cells fated to execute a specialized developmental function and subsequently die (Figure 1B). The observations that obsolete larval tissues in insects and amphibia undergo type 2 autophagic death during metamorphosis also support this premise [8,17]. It will be of interest to learn whether this presumed basis for non-apoptotic death can be extended to the suicide programs used by other cells that differentiate and carry out specific functions prior to their death.

Alternative mechanisms of cell death have been found to be important in certain pathological situations. Gain-of-function mutations in genes for $Ca^{2+}$ ion channel proteins lead to neurodegeneration in *C. elegans* by type 3 cell death [19]. HIV-1 kills CD4 T lymphocytes using a multiplicity of death programs, including type 3 cell death [20]. Several chemotherapeutic agents induce cancer cell death through the type 3 program [4]. A molecular understanding of alternative non-apoptotic cell death programs will be important for developing methods to intervene in pathological conditions associated with dysregulated cell death. In this regard, the *C. elegans* linker cell death system should prove useful as a model system to tease apart the mechanism of type 3 cell death.

The redundancy and diversity of cell death pathways underscore the robustness of this process. Robust occurrence of cell death, precisely on schedule, is essential for proper development and maintenance of life. The writer and Nobel laureate Rabindranath

Tagore echoed the consonance between life and death with the words "Death is not extinguishing the light; it is only putting out the lamp because the dawn has come". Future research into alternative cell death mechanisms should illuminate the diverse ways of 'putting out' the cell after it has accomplished its developmental goal.

**References**

1. Meier, P., Finch, A., and Evan, G. (2000). Apoptosis in development. Nature *407*, 796–801.
2. Kerr, J.F.R., Wyllie, A.H., and Currie, A.R. (1972). Apoptosis - basic biological phenomenon with wide-ranging implications in tissue kinetics. Brit. J. Cancer *26*, 239–257.
3. Lockshin, R.A., and Williams, C.M. (1965). Programmed cell death–I. cytology of degeneration in the intersegmental muscles of the Pernyi silkmoth. J. Insect Physiol. *11*, 123–133.
4. Edinger, A.L., and Thompson, C.B. (2004). Death by design: apoptosis, necrosis and autophagy. Curr. Opin. Cell Biol. *16*, 663–669.
5. Kroemer, G., and Martin, S.J. (2005). Caspase-independent cell death. Nat. Med. *11*, 725–730.
6. Abraham, M.C., Lu, Y., and Shaham, S. (2007). A morphologically conserved nonapoptotic program promotes linker cell death in *Caenorhabditis elegans*. Dev. Cell *12*, 73–86.
7. Mazzalupo, S., and Cooley, L. (2006). Illuminating the role of caspases during Drosophila oogenesis. Cell Death Differ. *13*, 1950–1959.
8. Clarke, P.G. (1990). Developmental cell death: morphological diversity and multiple mechanisms. Anat. Embryol. (Berl) *181*, 195–213.
9. Van Cruchten, S., and Van Den Broeck, W. (2002). Morphological and biochemical aspects of apoptosis, oncosis and necrosis. Anat. Histol. Embryol. *31*, 214–223.
10. Lettre, G., and Hengartner, M.O. (2006). Developmental apoptosis in *C. elegans*: a complex CEDnario. Nat. Rev. Mol. Cell Biol. *7*, 97–108.
11. Kimble, J., and Hirsh, D. (1979). The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev. Biol. *70*, 396–417.
12. Ellis, H.M., and Horvitz, H.R. (1986). Genetic control of programmed cell death in the nematode *C. elegans*. Cell *44*, 817–829.
13. McCall, K. (2004). Eggs over easy: cell death in the Drosophila ovary. Dev. Biol. *274*, 3–14.
14. Foley, K., and Cooley, L. (1998). Apoptosis in late stage *Drosophila* nurse cells does not require genes within the H99 deficiency. Development *125*, 1075–1082.
15. Witkus, E.R., Altman, L.G., and Taparowsky, J.A. (1980). An investigation of the presence of smooth endoplasmic reticulum and GERL during vitellogenesis in the ovary of Drosophila melanogaster. Exp. Cell Biol. *48*, 373–383.
16. Sulston, J.E., Albertson, D.G., and Thomson, J.N. (1980). The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. Dev. Biol. *78*, 542–576.
17. Yin, V.P., and Thummel, C.S. (2005). Mechanisms of steroid-triggered programmed cell death in *Drosophila*. Semin. Cell Dev. Biol. *16*, 237–243.
18. Soller, M., Bownes, M., and Kubli, E. (1999). Control of oocyte maturation in sexually mature *Drosophila* females. Dev. Biol. *208*, 337–351.
19. Syntichaki, P., Xu, K., Driscoll, M., and Tavernarakis, N. (2002). Specific aspartyl and calpain proteases are required for neurodegeneration in *C. elegans*. Nature *419*, 939–944.
20. Varbanov, M., Espert, L., and Biard-Piechaczyk, M. (2006). Mechanisms of CD4 T-cell depletion triggered by HIV-1 viral proteins. AIDS Rev. *8*, 221–236.

Department of MCD Biology, University of California, Santa Barbara, California 93106, USA.
E-mail: rothman@lifesci.ucsb.edu

DOI: 10.1016/j.cub.2007.02.032

# Morphogenesis: Joining the Dots to Shape an Embryo

**In the study of morphogenesis, how upstream signalling events are intricately linked to downstream cytoskeletal organisation is not entirely understood. Recent work in the *Drosophila* embryo has begun to shed light on this problem.**

**Sarah Woolner**

During the development of an embryo, its cells and tissues must be bent, tugged and sculpted into shape during numerous morphogenetic events. The general mechanisms used for morphogenesis are shared across the animal world and can be reactivated in adulthood to close a wound or during tumour metastasis. A key player in all morphogenetic events is the actin cytoskeleton, which in combination with non-muscle myosin II (actomyosin), can provide the contractile force to shape a single cell or a whole tissue.

Various green fluorescent protein (GFP)-based tools now allow for the observation of changes in the organisation of actin, myosin and cell adhesions in the lead-up to and during morphogenetic events [1–3]. Genetic studies have also revealed a great deal about the upstream events that are required for morphogenesis, such as local signalling pathways or the transcription of specific morphogenetic regulators. However, the biggest gaps in our knowledge concern the links between these upstream events and the downstream changes in the organisation of actomyosin and cell-cell adhesions. Bridging these gaps will be crucial to gaining a complete understanding of how tissues and organs are shaped during development. Recent work using *Drosophila* embryogenesis as a model system has made sizable steps towards doing just that [4–7].

One well studied example of morphogenesis is gastrulation, the process whereby the different cell layers of the embryo are laid down. In the *Drosophila* embryo, gastrulation begins with the invagination of a group of cells on the ventral surface of the embryo, forming the ventral furrow (reviewed in [8]). The cells of the ventral furrow are internalized and will later undergo an epithelial to mesenchymal transition to form the mesoderm precursors of the embryo. Ventral furrow formation is driven by the apical constriction of the ventral cells, which causes them to change from cuboidal to wedge-shaped — a transition that forces them inside the embryo (Figure 1A). The apical